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Inhibition of DNA Replication Fork Progression by Topoisomerase-targeted Antileukemia Agents

Many potent chemotherapeutic agents are available for the treatment of human leukemias. However, large gaps still persist in our knowledge concerning the factors that determine the therapeutic success or failure in the patients. This is in part due to the limited number of studies that investigate the biochemical and molecular mechanisms that lead to arrest of leukemia cell growth and cell death following exposure to the chemotherapeutic agents. Very effective antileukemia agents, including doxorubicin, daunorubicin, amsacrine, etoposide, teniposide and camptothecin, interfere with the activity of DNA topoisomerases. Two classes of topoisomerases have been identified. Both topoisomerase I and topoisomerase II appear to be essential for replication and survival of the leukemia cells. DNA topoisomerases allow the progression of the replication complexes along the template DNA and the separation of the replicated DNA molecules. The overall goal of this research project is to provide insights into the mechanisms by which certain topoisomerase I- and topoisomerase II- targeted antileukemia agents block DNA replication and proliferation of leukemia cells. It is proposed that these agents trap the topoisomerases in a complex with the DNA. The hypothesis that will be tested in the proposed studies is that replication complexes moving along the template DNA collide with the drug-topoisomerase-DNA complexes. The collision would arrest the progression of the replication complexes and generate irreversible DNA damage. This would ultimately lead to arrest of the growth and death of the leukemia cells. The applicant plans to investigate this hypothesis by using techniques of cellular and molecular biology. It will be determined how the formation of drug-topoisomerase-DNA complexes block the replication of an oncogene (*c-myc*) in human leukemia cells and how irreversible damage is generated in replicating DNA.

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Cloning and Characterization of TcRζ/CD3:p59$^{fyn(T)}$ Associated p120/130

Many oncogenes (cancer causing genes) have been implicated in the control of cell division and malignancy. The p59$^{fyn(T)}$ protein-tyrosine kinase, a member of the src family of protooncogenes, has been shown to be associated with the antigen receptor (TcRζ/CD3) complex of T lymphocytes. Therefore it is likely to be involved in the mediation of important activation mechanisms that are generated when T lymphocytes encounter antigens. We have recently reported the identification of a p120/130 tyrosine substrate that appears to be specifically recognized in association with p59$^{fyn(T)}$ (but not p56^{lck}, another src-like kinase expressed in T cells, which associates with CD4 and CD8). Importantly, p120/130 can physically associate with the TcRζ/CD3 complex via the fyn kinase. Thus p120/130 may be a fyn specific substrate in T cells and could therefore serve as a marker for the signals generated by the kinase during T cell activation via the antigen receptor. Moreover, given the p120/130 is likely to mediate key functions in the activation of T cells, itself may possess oncogenic properties. We have purified this protein and subjected it to partial internal peptide sequencing. Using degenerate oligonucleotides and anti-p120/130 antisera to screen T cell derived cDNA libraries, we are currently attempting to isolate a cDNA clone encoding p120/130. In this proposal we seek to continue our efforts to obtain a full length cDNA clone and proceed towards its biochemical and functional characterization. Transfection studies with T cell clones will be carried out in an attempt to determine whether overexpression of p120/130 can induce transformation. Finally, p120/130 specific reagents would eventually provide important tools for the study of T cell malignancies in order to find a correlation between the mechanisms mediating T lymphocyte activation and transformation.